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# OLEIC ACID ABSORPTION FROM INTESTINAL ALLOGRAFTS

BY

© KENG EOK GOH

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH
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FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the faculty of Graduate Studies and Research, for acceptance, a thesis entitled:

OLEIC ACID ABSORPTION OF INTESTINAL ALLOGRAFTS

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in partial fulfilment of the requirements for the degree of M.Sc. in Experimental Surgery.

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## ABSTRACT

Mongrel dogs of approximately equal weight were autotopically allografted using small intestine. The intestinal segments were then converted into Thiry Vella fistulae. Post-operatively, these dogs received Prednisone, Imuran and Antilymphocyte sera routinely for immunosuppression.

The dogs were studied at one week and three weeks after surgery by introducing 250 microcuries of $H^3$-9-10 oleic acid into each Thiry Vella fistula and the absorption followed by measuring the plasma radioactivity each hour for eight hours.

The absorption of the oleic acid was also followed by measuring the thoracic duct lymph radioactivity. The results from these dogs were then compared with those of untransplanted and autotransplanted dogs with Thiry-Vella fistulae at one and three weeks after surgery. Statistical analysis indicated that absorption of the oleic acid did not differ in the three groups of dogs. Immunosuppression did not affect the absorption significantly and a great proportion of the absorbed oleic acid appeared to enter the vascular system directly. Furthermore, the oleic acid appeared in the thoracic duct of the allografts and autografts as early as one week after surgery indicating that lymphatic regeneration had already occurred.

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## LITERATURE REVIEW

### 1. Introduction.

Massive resection of small intestine has been performed for various reasons, the most common being mesenteric vascular accidents, massive volvulus, strangulated internal hernias, and generalized inflammatory disease of the small intestine (Wright & Tilson, 1971). The complications subsequent to massive resection, however, still cannot be entirely avoided. A great proportion of these people with massive resections develop the characteristic "short gut syndrome", with acidic diarrhea leading to fluid and electrolyte imbalance, steatorrhea and undernutrition due to their inability to absorb fat, carbohydrates and proteins (Wright & Tilson, 1971). The development of gross steatorrhea also leads to severe loss of calcium and magnesium, since these minerals form insoluble soaps with fatty acids, and thus cannot be absorbed (Scott, 1968). This is aggravated by an inability of these patients to absorb the fat soluble vitamin D which normally aids calcium absorption from the intestine. Severe calcium and magnesium deficiency can arise in such cases, leading to the development of tetany, osteoporosis and psychic disturbances such as depression (Scott, 1968). Other important disturbances following massive resection of small intestine include vitamin $B_{12}$ deficiency due to loss of the distal ileum, and deficiency of the other fat soluble vitamins A and K. All these deficiencies produce serious consequences.

The treatment of such patients can be directed along two lines. Firstly, it can be directed at replacement of fat, carbohydrate, protein, vitamin and minerals by oral or intravenous means (Wright & Tilson, 1971; Thompson, et al, 1969; Zurier, 1966), and increasing the absorptive ability of the remaining intact intestine by various methods including reversal of segments of small intestine (Fink and Olson, 1967), and use of a constipating agent such as codeine (Kinney, et al, 1962). These methods are attended by some serious complications (Sedgewick & Viglotti, 1971; Freeman & MacLean, 1971), and have only limited success. Secondly, treatment can be directed at replacement of the lost bowel by transplantation. This, if it can be done successfully, would restore the normal absorptive capacity of the individual and free him from the long dreaded course of replacement therapy. Such treatment is only in the experimental stage, although intestinal transplantation has been carried out in animals since 1959.

Several investigators have demonstrated very clearly the feasibility of long term survival of intestinal allografts (Jordan, 1971; Lillehei, et al, 1967; Preston et al, 1965; State, 1969). Jordan obtained an average survival time in allografts of 80 days using Prednisone, Imuran (Azathioprine) and Antilymphocyte or antimacrophage sera. Initial tests on these allografts showed that they functioned normally. Thus, analysis of the secretions of intestinal allografts showed that the concentrations of sodium, potassium chloride and bicarbonate resembled those in

isolated intestinal fistulae (Preston, et al, 1965). Furthermore, (State, 1969 & Ruiz et al, 1972) found that these allografts could actively absorb 14-c-d-arabinose, 14-c-d-glucose and D-xylose. Studies in our own laboratory have shown that these allografts also absorb d-alanine normally (Moisey, 1971). The ability to absorb fat has yet to be determined. Preliminary studies in our laboratory by an optical density method showed that some olive oil was absorbed by these allografts (Dikman, 1968). The purpose of our investigation is to compare the absorption of oleic acid by canine small intestinal allografts with that of normal, untransplanted and autotransplanted small intestine.

## II. Historical Review.

The first observation on fat absorption was made by Olaf Rudbeck in 1653. He noted the milky appearance of the thoracic duct after a fat meal (Rudbeck, 1942). Two hundred years later, Claude Bernard (1856) observed that lymphatics just distal to the pancreatic duct of rabbits became milky after a fatty meal. He proposed the involvement of pancreatic juice in this process. This laid the foundation for development of the Particulate Theory, which postulated that finely emulsified fat droplets simply filtered through tiny pores in the intestinal surface. Hoppe-Seyler (1877) agreed, but proposed that fat was partly split by pancreatic lipase to glycerol and fatty acids to form soaps stabilized by bile. The soaps then passed directly into the lacteals. Munk (1880), convinced by his own observations that free fatty acids or glycerides of fatty acids could increase triglycerides in the lymph, became the principle proponent of the Particulate Theory.

At the turn of the century, Pfluger (1900a, 1900b, 1900c) questioned the Particulate Theory of absorption, and became the champion of Lipolytic Theory. He maintained that fats were completely hydrolysed to glycerol and fatty acids which formed soluble soaps and were absorbed. The Lipolytic Theory prevailed for more than 40 years and was well set forth by Bloom (1943), Verzar and McDougall (1936). The latter further suggested that bile played an important part in the stabilization of fatty acids.

In 1938, Fraser (1938) raised doubts about the Lipolytic Theory with his observations on emulsions. He noted that fat was not completely hydrolyzed but monoglycerides and diglycerides were formed as well (Fraser et al, 1944), and that monoglycerides, fatty acids and bile salts formed very stable complexes (Fraser & Sammons, 1945). He therefore proposed a Partition Theory, maintaining that the latter complexes stabilized emulsions of diglycerides and triglycerides, a fact that permitted their passing into the intestinal cells through "pores" or "canals" described by Baker (1942). The glycerides pass into the lymphatic circulation while the fatty acids appear in the portal venous blood. The experimental facts are summarized in his reviews (Fraser, 1946; Fraser, 1958).

The Partition Theory prevailed for more than a decade until Bloom et al (1950, 1951a, 1951b), using radioactive labels, demonstrated that 80 to 90 percent of ingested long-chain fatty acids could be recovered in the lymph of rats as triglycerides. Fatty acids with two to ten carbon atoms were transported predominantly through the portal system.

Developments of the last 22 years have clarified the situation. Studies with the electron microscope (Granger, 1950; Palay, 1959a; Palay, 1951b) demonstrated that the idea of absorption of fat as emulsions was untenable. The specificity of pancreatic lipase action on triglycerides was demonstrated by Mattson et al, 1956 and Desnuelle and Savary, 1956;1963.

Following this, Hofman and Borgstrom then forwarded the concept of Micelle formation and its relationship to fat absorption (Borgstrom, 1962; Hofman and Borgstrom, 1962; Hofman, 1966).

III The Absorption of Oleic Acid.

The major portion of the average North American's dietary fat consists of triglycerides made up of long chain fatty acids (Schiff & Dietschy, 1969; Hofman & Borgstrom, 1962), of which oleic acid is a member. These fatty acids are formed from the hydrolysis of triglycerides in the intestinal lumen (Senior, 1964; Johnston, 1968). The absorption of oleic acid, like all other long chain fatty acids, consists of the following steps:

1. Solubilization.
2. Cellular Penetration.
3. Cellular Phase.
    a. Esterification
    b. Chylomicron Formation.
4. Transport from the cell.

1. Solubilization:

(a) Effect of Bile Salts.

Oleic acid, like all lipids, is notoriously insoluble in water. The absorption of these lipids thus involves the conversion of the normally water insoluble form to a water soluble state (Hofman, 1966). This is mediated by the action of conjugated bile salts, which contain both a polar and a non polar end in each molecule (Roepke and Mason, 1940; McBain, 1942; Hartley, 1955). Molecules of this type have been termed amphipathic (Hartley, 1936), and are normally water insoluble. However, when their concentration

exceeds a certain critical level, termed the critical micellar concentration (CMC) Roepke, 1940), and the temperature exceeds a critical point, the Kraft point (CMT) (Garrett, 1961), they form small molecular aggregates called micelles (Hartley, 1936; Osipow, 1962; Shinoda, 1963), and become soluble in aqueous solution. A micelle is a spherical aggregate of bile salt molecules, each molecule oriented with non polar groups in the centre and polar groups toward the surface of the sphere (Hofman, 1962; Hofman, 1965; Hofman, 1968). The size of the aggregates has been estimated on Sephadex columns by Borgstrom and found to be approximately 16-20 Angstroms in diameter (Borgstrom, 1965). Each has an anhydrous molecular weight of approximately 25,000 representing about eight-five bile salt molecules in each micelle (Dawson, 1967). Hofman (1963), found that the critical micellar concentration of a physiological mixture of bile salts, under conditions simulating those in the intestinal lumen, is 2-3 milliMoles per liter (Hofman, 1963). This is far below the concentrations normally found in the human bile (50-150 milliMoles) and intestinal contents (60-100 milliMoles) (Hofman, 1965). Thus, under physiological conditions, bile salts are in micellar solution. When polar lipids, like oleic acid, come into contact with these micelles, they dissolve in the micelles in such a way that their polar heads are on the outer surface of the spherical micelle, and their non polar heads are in the interior of the micelle (Hofman, 1965). This process has been

termed solubilization (Hofman, 1962; Hofman, 1965).

(b) Effect of Monoglycerides.

Monoglycerides aid fatty acid solubilization by expanding the bile salt micelle, thereby allowing more lipid to solubilize in the micelle (Hofman, 1962). This has been termed enhanced solubilization (Hofman, 1962).

(c) Effect of pH.

The micellar solubility of fatty acids is dependent on the pH of the medium. Hofman (1966) demonstrated that ionized fatty acids have greater solubility than unionized fatty acids in bile salts. Ionization of all long chain fatty acids (Hofman, 1964) takes place when the pH exceeds 6.1 and ionization increases with pH.

Saturation also affects the solubility of fatty acids. Lawrence (1964), demonstrated that unsaturated fatty acids have better micellar solubility than saturated ones. Thus, oleic acid, being unsaturated, has better micellar solubility than a saturated long chain fatty acid like palmitic acid.

The importance of micelle formation is evident. Formation of micellar solutions very effectively reduces the size of the lipid from 300-1000 millimicrons in an emulsion to 16-20 Angstroms. Such a reduction in size greatly enlarges the surface area of the lipids that come into contact with the absorptive surface of the mucosal cell, and thus facilitate the diffusion process into the cell. Johnston and Borgstrom (1964), have also shown that hamster brush

border took up much more oleic acid in micellar solution than albumin bound or emulsified oleic acid.

Despite the strong evidence supporting the importance of micelles in fatty acid absorption, a species difference exists. Several investigators have demonstrated some lipid absorption in bile deficiency states. Annegers (1954), and Cohen (1961), by following fecal fat excretion, found that dogs with bile fistulae could assimilate approximately 40% of fed oleic acid. Gallagher et al (1965), introduced $14_C$ oleic acid into the duodenum of rats with bile fistulae and found that up to 65% was absorbed over a 12 hour period compared to approximately 82% in control rats.

2. Cellular Penetration.

Following solubilization, fatty acids enter the mucosa. Originally, it was maintained that they enter by a process of pinocytosis (Palay, 1959a; 1959b; Ashworth, et al, 1965; Palay, 1964; Saunders & Ashworth, 1961; Strauss, 1963). Recent biochemical (Johnston and Borgstrom, 1964; Strauss & Ito, 1965) and morphologic (Phelps, et al, 1964; Taylor and Adamstone, 1965; Lacy and Taylor, 1962; Rostgaard and Barrnett, 1965; Cardell, et al, 1967) evidence however, strongly favours a process of passive diffusion across the plasma membrane. Thus, pinocytosis was noted only infrequently during lipid absorption, too rarely to account for total lipid transport (Phelps, et al, 1964; Taylor and Adamstone, 1965; Lacy and Taylor, 1962; Rostgaard and Barrnett, 1965; Cardell, et al, 1967). In addition, electron microscopy showed that pits

between the microvilli, previously believed to function in the transport of lipids, were not involved in lipid absorption (Cardell et al, 1967). Finally lipid absorption was found to be energy independent (Johnston and Borgstrom, 1963; Strauss and Ito, 1965), whereas pinocytosis was energy dependent (Rustad, 1964).

The exact role of micelles in this transport process is still controversial. Some observers claim the transport of the whole micelle into the cell (Lacy and Taylor, 1962; Rostgaard and Barrnett, 1965; Johnston and Borgstrom, 1963; Barrnett and Rostgaard, 1965; Gordon and Kern, 1968; Rostgaard and Barrnett, 1964), while others (Senior, 1964; Johnston, 1968; and Thornton et al, 1968), maintain that the micelles act only as carriers of fatty acids and monoglycerides to the surface of the cell.

Regardless of the exact mode of transport into the cell, bile salts do not remain on the cell surface, since they are absorbed at a site further down in the small intestine (Booth, 1967). This difference in absorption site serves a useful purpose as it allows bile to remain at the site of lipid absorption and participate in further solubilization of lipids (Senior, 1964; Johnston, 1968). These bile salts later pass to the distal ileum where they are absorbed, carried to the liver and are released again in the bile, thus completing the "enterohepatic Circulation" (Lack and Weiner, 1963; 1967; and Dietschy, 1968). It has been estimated that the total bile salt pool circulates in

this manner twice during a normal meal in order to provide adequate bile salts for solubilization (Shinoda et al, 1963; Hofman, 1963).

3. Cellular Phase.

(a) Esterification.

Once within the cell, oleic acid is activated to its CoA derivative by the enzyme, fatty acid: CoA lipase (AMP) [6.2.1.3.] (Clark & Hubscher, 1960; 1961; Dawson and Isselbacher, 1959). This enzyme has also been named acyl CoA synthetase and fatty acid thiokinase (Senior, 1964; Johnston, 1968; Report, 1961), and has a specificity for long chain fatty acids (Dawson and Isselbacher, 1960a). This process takes in the smooth endoplasmic reticulum (Clark and Hubscher, 1960; 1961; Dawson and Isselbacher, 1959; Senior and Isselbacher, 1960; Dobbins, 1969). Activated oleic acid then esterifies the absorbed monoglycerides to diglycerides by the action of the enzyme acyl CoA: monoglycerides acyl transferase (Senior, 1964; Ailhaud et al, 1964). Diglycerides formed this way and from the alpha-glycerophosphate pathway (Senior, 1964), are then esterified to triglycerides by the activated fatty acid and the enzyme acyl CoA: diglyceride acyl transferase (Senior, 1964; Dawson and Isselbacher, 1960). This whole process of esterification again takes place in the smooth endoplasmic reticulum (Clark & Hubscher, 1960; 1961; Dawson and Isselbacher, 1959; Dobbins, 1969; Strauss, 1966).

(b) Chylomicron Formation.

Triglycerides formed by the above process, acquire an

envelope of phospholipid, protein and cholesterol to form chylomicrons (Senior, 1964; Johnston, 1968; Dobbins, 1969). The mechanism of this is not well understood. Studies show that chylomicrons and two percent proteins (Johnston, 1968; Zilversmit, 1967) consist of 86 percent triglycerides of the long chain type, 8.5 percent phospholipid, three percent cholesterol and cholesterol esters. Each has a diameter of 0.2 - 0.3 microns and are formed in the smooth endoplasmic reticulum. Its formation depends on betalipoprotein synthesis in the cell. This has been shown by Isselbacher et al (1963) and Sabesin et al (1965) using betalipoprotein inhibitors puromycin and acetoxycycloheximide. When these investigators gave either of these substances to rats, they found that chylomicron formation was inhibited and impaired lipid absorption resulted. Lipoprotein dependence is also indicated by the observation that betalipoprotein deficiency also results in defective lipid absorption (Sabesin and Isselbacher, 1965).

Chylomicrons thus formed are then stored temporarily in the Golgi apparatus, (Dobbins, 1969). Release of chylomicrons from the cell presumably occurs by a process of reverse pinocytosis (Dobbins 1969). Membranes of the Golgi vacuoles fuse with the lateral plasma membrane of the cell, then open to the extracellular space and discharge their contents into it. However, this process has never been convincingly demonstrated (Dobbins, 1969). Once in the extracellular space, the chylomicrons pass through the basal lamina of the mucosal cell and wander through the lamina propria to enter lacteals via gaps between endothelial cells (Dobbins, 1969). The passage of chylomicrons

into the lacteals has been described by several investigators (Palay and Karlin, 1959b; Ashworth and Sternbridge, 1960; Clark, 1959; Thomas and O'Neal, 1960; Casley-Smith, 1962). Session et al (1968), elegantly summarizes it in the following way: ".....when the villus initially relaxes, the lacteal is momentarily empty and the higher pressure in the interstitial tissue causes dislocation of the endothelial cells toward the lumen, opening of the junctional areas, and permitting flow into the lacteals. Once the lacteal is full, the villous muscle contracts, and endothelial cells are forced outward against the connective tissue, thus closing the intercellular junction". The flow of lymph carrying the chylomicrons results from the contraction and relaxation of the muscularis mucosae. Some evidence suggests that the hormone villikinin controls this contraction by the villous muscles (Sessions, 1968; Kokas and Johnson, 1965).

4. Transport From the Cell.

The classic experiments of Bloom et al with 14-C labelled fatty acids and thoracic duct cannulation in rats, indicate that the route of transport of fatty acids from the cell depends on the chain length of the fatty acid. Those with more than twelve carbon atoms such as oleic acid, leave the intestine chiefly by the lymphatic system (Bloom et al, 1950b; 1951b; Chaikoff et al, 1951; Bloom, 1951a). Short and medium chain fatty acids with less than twelve carbon atoms enter the portal vein directly without esterification (Bloom et al, 1951b). However, this separation is not absolute.

Dawson, (1967), cautioned that it may be variable and quite unpredictable. Several investigators have shown that between 25-30% of long chain fatty acids are normally transported by the portal route (Gallagher et al, 1965; Dawson et al, 1964; Hyun et al, 1967). This route is presumably taken by any long chain fatty acids which escape esterification (Dawson, 1967). In animals with defective esterification, the portal route plays a major role in the transportation of long chain fatty acids (Koldovsky et al, 1963). In rats with biliary fistulae, only a small proportion of absorbed, unesterified, long chain fatty acids enter the lymphatic system (Gallagher et al, 1965; Borgstrom, 1953; Dawson, 1960b). The esterification process thus seems to be an essential step for lymphatic transport (Dawson, 1967).

5. Site of Fatty Acid Absorption.

This varies somewhat from one species to another. Studies in man show that, the proximal jejunum serves as the major site of lipid absorption. Thus, in patients with bowel resections, most of the dietary fat can still be absorbed when the proximal six feet of small intestine is preserved (Booth et al, 1961b). Borgstrom, et al, (1957), found that the proximal one hundred centimeters of small intestine absorbed ninety to ninety-five percent of fed corn oil.

In dogs, however, the situation is not as clear. Kremen, et al, (1954), found that loss of the distal fifty percent of the small intestine caused a profound increase in fecal fat and a fifteen

percent weight loss, whereas this did not occur in dogs with the proximal fifty percent of the small intestine resected. They concluded that the distal small intestine was the major site of lipid absorption. The defective lipid absorption in this case, however, may be due to interruption of the enterohepatic circulation of the bile which is absorbed in the distal ileum, and consequent deficient micellar formation (Lack and Weiner, 1953). More recent work by Turner (1958), using $I^{131}$ labelled oleic acid and triolein, show that the distal jejunum and proximal ileum absorb most of these substances. However, an area specifically adapted for lipid absorption probably does not exist (Johnston and Borgstrom, 1964; Bennet, 1964), and with a large intake of lipids, absorption also occurs in the rest of the ileum in both animals (Booth et al, 1961a; Hoving and Valkema, 1969), and man (Booth, et al, 1961b).

6. Factors Interfering with Oleic Acid Absorption.

The absorption of the oleic acid can be affected in several ways.

1. Interference with Micelle Formation.

Firstly, conjugated bile salts have to be present in concentrations exceeding the critical micellar concentration in order to solubilize fatty acids. Failure to provide a critical micellar concentration of bile interferes with solubilization and absorption of long chain fatty acids.

Lack of monoglycerides decreases oleic acid solubility and

therefore, its absorption.

Finally, failure to produce ionization of oleic acid by too low a pH would interfere with its solubility in bile and thereby interfere with its absorption.

2. Interference with Oleic Acid Diffusion.

Since the transport of oleic acid into the mucosal cell is by simple diffusion, its rate of absorption would vary directly with the surface area of the intestinal epithelium. Thus, any factor which reduces the intestinal epithelium could reduce the absorption of oleic acid. Azathioprine, by increasing epithelial cell loss (Frei, 1967), could conceivably reduce fatty acid absorption. This however, has not been demonstrated experimentally. By the same token, graft rejection causing sloughing of the superficial layers of the intestinal epithelium interferes with oleic acid absorption.

3. Interference of Chylomicron Formation.

Any factor which interrupts chylomicron formation will reduce absorption of long chain fatty acids. Thus inhibition of lipoproteins which is necessary for chylomicron formation hampers absorption of long chain fatty acids as shown by Isselbacher et al, 1963; Sabesin et al, 1965, using B-lipoprotein inhibitors like Puromycin and Acetoxycycloheximide.

4. Interruption of Chylomicron Transport.

Since the main portion of chylomicrons are transported by the lymphatics, interruption of lymphatic channels, such as in

intestinal transplantation, would also interrupt the transport of the main portion of absorbed long chain fatty acids (Bloom et al, 1950b; 1951a; 1951b; Chaikoff et al, 1951).

## METHOD AND MATERIALS

### I. Animals.

Healthy, adult, mongrel dogs of both sexes, weighing between 15 - 25 kilograms were obtained from the University farm. These were quarantined and observed for two weeks to ascertain that they were free from disease such as distemper, before coming to the laboratory.

### II. Preparation of Antilymphocyte Serum (Jordan, 1971).

Goat anti-dog lymphocyte serum was prepared by injecting goats intraperitoneally with 20 million lymphocytes (prepared as indicated below) in Freund's complete adjuvant, followed by a similar injection two weeks later. The goats were bled from the external jugular vein two weeks after the second injection. Five-hundred Milliliters of blood were obtained at each bleeding. The goats were given monthly booster injections and bleeding was done two weeks after each booster. Blood obtained in such a manner was refrigerated overnight and allowed to clot. It was then centrifuged to separate the serum which was removed into a beaker. The serum was decomplemented by heating to 56°C for 30 minutes. Hemaglutinins were removed by absorption with dog erythrocytes, using two volumes of the latter with one volume of serum at 37°C for 30 minutes. This mixture was centrifuged and the serum was pipetted off into tubes and frozen until ready for use.

### III. Preparation of Lymphocyte Suspension.

Mesenteric lymph nodes were obtained from dogs used in other acute experiments. All traces of fat and connective tissue

were carefully removed from the nodes, which were suspended in Ringer's lactate solution, and then homogenized. The suspension was filtered twice through fine cheesecloth, washed in Ringer's lactate solution, and the cells were suspended in the latter. Lymphocyte counts per milliliter were done on this solution.

IV. Testing of the Antilymphocyte Serum.

Antilymphocyte serum was tested by a modified leucoagglutination test of Amos and Peacock and Gray et al, 1967 and by the cytotoxic test as previously done in this laboratory (Jordan, 1971).

V. Preparation of Test Solution.

A modified method of Johnston and Borgstrom (1964) was adopted. Oleic 9-10-H-3 acid was obtained from New England Nuclear, Dorval, Quebec. This came in quantities of five millicuries dissolved in 0.5 milliliter (ml) of hexane. It had a molecular weight of 282.5 and a radiopurity greater than 99 percent. Five millicuries were dissolved in heptane to obtain a concentration of five millicuries per 100 ml. The solution was stored as such and the radioactivity was checked periodically to ascertain that the concentration was constant. 250 microcuries or (5ml) of the stock solutions were pipetted into a beaker containing 10 milligrams (mg) of unlabelled 84% oleic acid which acted as a carrier. This was agitated to dissolve the latter. The heptane was then evaporated over a water bath, evaporation being considered complete when the odor of heptane was gone. A 20 ml micellar solution containing 10 milliMoles (mM) of sodium taurocholate in normal saline and 10 mg monolein# was

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# Coleman and Bell, Manufacturing Chemist, Los Angeles, California.

prepared. The pH was found to be usually 5.7 - 5.8. This was raised to a pH of 7 - 7.5 with sodium hydroxide to obtain ionization of the oleic acid and increase its solubilization (Hofman, 1966). This solution was then added to the oleic acid and the mixture agitated gently to form a mixed micellar solution. 0.5 ml of this was removed to count for radioactivity and the total amount of radioactivity in the test solution verified by accurately measuring the volume of the solution. The radioactive concentrations of such solutions were checked periodically and found to be constant, assuring us that they were homogenous. Only freshly prepared solutions were used for our tests. No solution was prepared more than 24 hours before each test. The test solutions remained clear even after standing for 3 days and their radioactivity also stayed the same (within 5%) as that of the freshly prepared solution, indicating that these solutions were stable for at least three days after preparation.

VI. Preoperative Management.

Group I dogs (below) were given 5 ml of antilymphocyte serum subcutaneously daily for three days prior to surgery. All dogs were fasted one day prior to surgery.

VII. Operative Management.

In a typical experiment, two dogs were anesthetized with intravenous Diabutal* using 0.5 ml per kilogram of body weight. An endotracheal tube was then inserted. The animals were divided into

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* Sodium Pentobarbital - 60 mg per ml.

the following groups:

Group I: Allograft. (Figure I).

Dogs were matched for weight only. They were opened through a midline abdominal incision and splenectomy was performed through the distal vascular arcades near the splenic capsule. The small intestine from approximately 50 cm distal to the Ligament of Treitz to a point 10 cm proximal to the ileo-cecal junction, was isolated. The superior mesenteric artery and vein supplying this were exposed by transecting the surrounding lymph nodes and dissecting intervening connective tissue. The freed vessels were then clamped with disposable plastic vascular clamps, and transected cleanly. The whole segment of intestine was then taken out of the abdomen and perfused through the artery with cold dog plasma. The prepared intestines were then exchanged between animals. Vascular continuity was re-established with a single continuous layer of 7-0 silk suture to approximate the transected ends of the graft and host mesenteric vessels. Continuity of the lymph nodes was re-established with 4-0 silk sutures, and that of the dog's own intestine with an end-to-end anastomosis using a single layer of interrupted 4-0 silk sutures. The ends of the allograft were brought out on each side of the abdomen through separate stab incisions to form a Thiry-Vella fistula (Parson, 1968).

Group II: Autotransplants. (Figure 2).

In this group, the same procedure was done as with Group I animals, except that the isolated intestinal segment was not

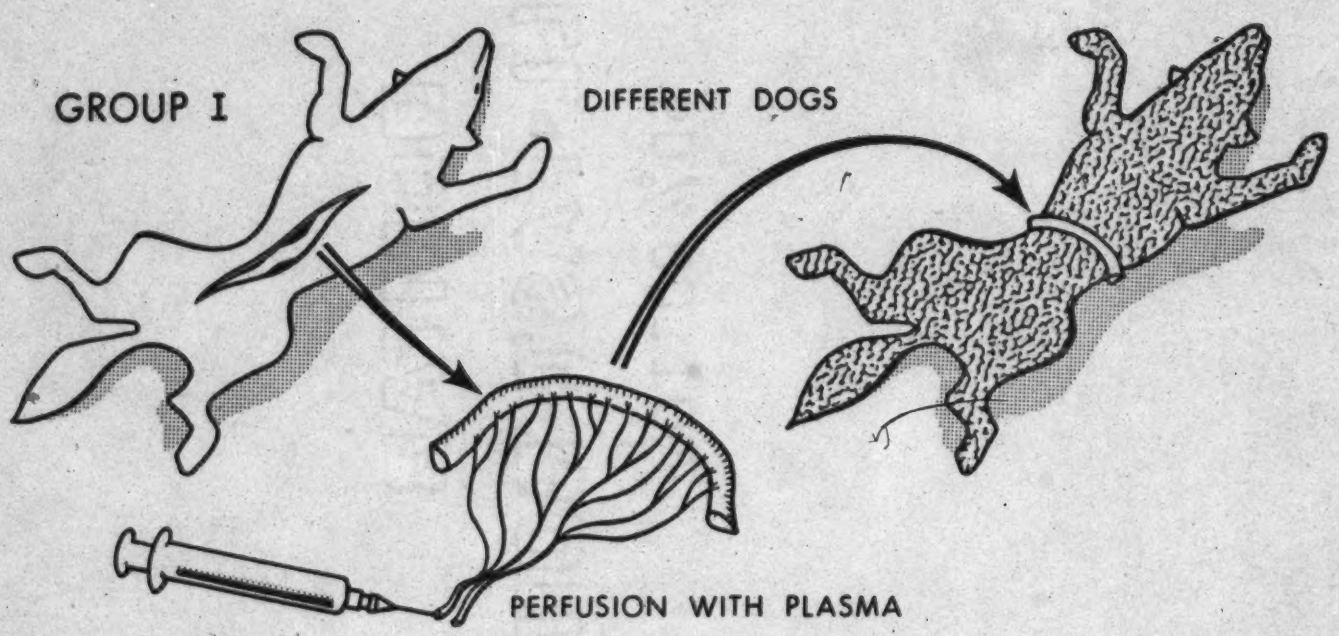


Figure 1. Allograft. Segment of small intestine removed from each dog, perfused with pooled plasma and exchange transplanted into partner. Each allograft is brought out as a Thiry-Vella fistula.

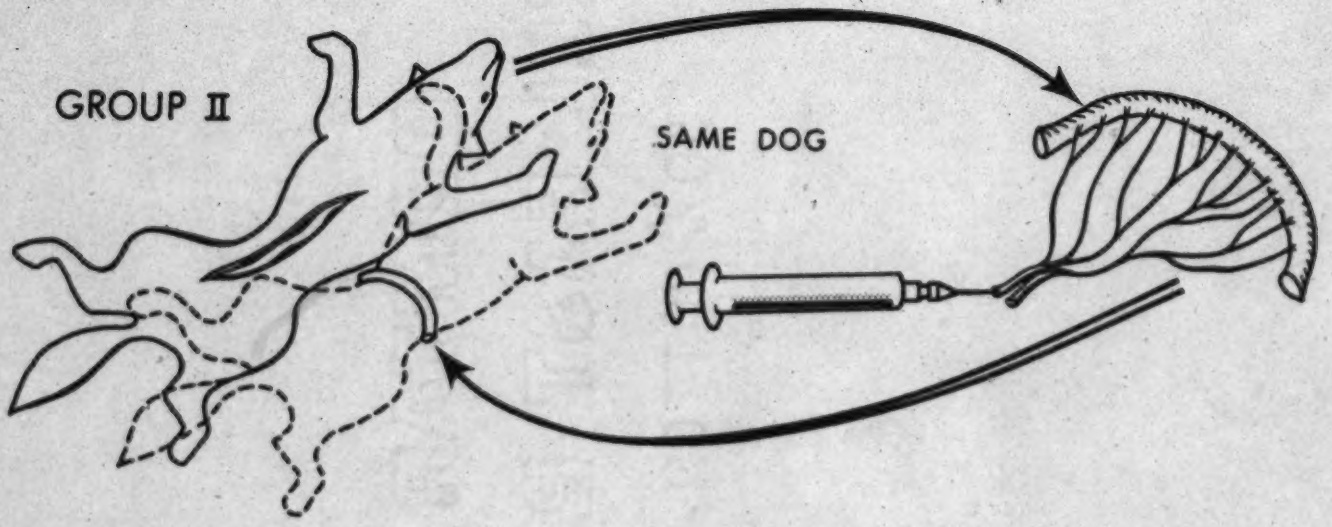


Figure 2. Autotransplant. Segment of small intestine removed from each dog, perfused with pooled plasma, autotransplanted and established as a Thiry-Vella fistula.

exchanged with its partner, but was implanted back into the same dog.

Group III: Control Thiry-Vella Fistula Dogs. (Fig. 3).

In this group, after splenectomy, the small intestine was isolated without interfering with the nutrient vessels or the lymph nodes. The segment was then made into a Thiry-Vella as in groups I and II animals.

The anoxia time of the grafts in Group I and II was between 20-40 minutes. There was no anoxia with Group III animals. The abdomen in each case was closed with a single layer of 1-0 chromic interrupted sutures on the fascia and 3-0 silk vertical mattresses on the skin.

VIII. Post Operative Management.

Dogs in group I-III were given nothing by mouth for the first two days postoperatively, except for immunosuppressive drugs. They were maintained on 1500 ml of five percent dextrose/normal saline solution intravenously daily until the third day when they were started on liquid diet, progressing gradually to a full diet over a period of a week. Group I dogs received 20-25 mg of Prednisone*, 150-200 mg of Imuran** orally and 5 ml of antilymphocyte serum subcutaneously daily starting on the day of surgery. These were gradually reduced after three weeks to a maintenance dose of 10 mg of Prednisone, 50 mg of Imuran and 5 ml of antilymphocyte serum three times a week.

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* British Drug Houses Ltd., Toronto, Canada
** Azathioprine (Burroughs Wellcome and Co.)

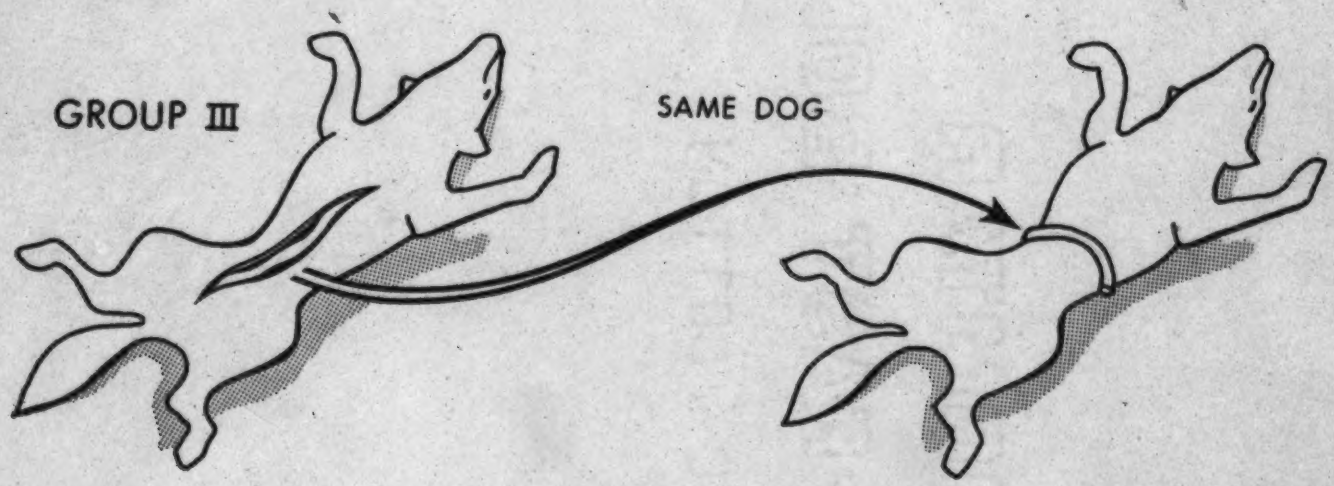


Figure 3. Control Thiry-Vella fistula. Segment of small intestine isolated without transacting the vessels or lymph nodes and brought out as a Thiry-Vella fistula.

Figure 4. Thoracic duct cannulation. Photograph shows a 1/8 inch diameter Silastic cannula entering the superior lymphatic, past the ampulla into the thoracic duct. The connection between the ampulla and the jugular vein has been ligated.

IX. Thoracic Duct Cannulation (Figure 4).

This method was first described by Markowits (1954) and modified in this laboratory by Friedman (1956). The latter cannulated the thoracic duct through a hypodermic needle. This enabled him to insert only a small cannula into the duct and thus he encountered a great deal of problems with clotting. Therefore, a slight variation of the procedure was adopted by us to allow us to use a larger cannula.

Each dog was fed a fatty meal consisting of milk and cream two hours before surgery. It was anesthetized with Diabutal intravenously, an endotracheal tube was inserted, and the animal was restrained in a supine position with the neck fully extended. A three inch horizontal incision was made in the neck extending from the midline, two centimeters above the sternum, to the left shoulder tip. Dissection was carried down to the external jugular vein which was then freed down to its junction with the subclavian vein. The superior lymphatic, appearing as a thin white structure, lay posteromedial to the external jugular vein. Following this lymphatic inferiorly, the ampulla of the thoracic duct was located entering the posterior wall of the jugular vein at the junction of the latter with the subclavian vein. The superior lymphatic and the ampulla were sometimes collapsed and difficult to identify, the only indication of their presence being the periodic gush of venous blood into them. A ligature was placed around the ampulla and tied

Figure 5. The photograph shows the left opening of a Thiry-Vella fistula occluded by an external balloon around the Foley catheter that has been inserted into the opening and held in place by a similar balloon deep in the opening.

at its entrance into the jugular vein. This caused the ampulla, thoracic duct and superior lymphatic to dilate enormously. The superior lymphatic was ligated two centimeters above the ampulla and an anchoring suture was placed on each side of its wall at a point one centimeter above the ampulla. This produced tenting of the duct and allowed it to be opened easily by a small longitudinal incision. The anchoring sutures held open this incision and permitted easy cannulation with a Silastic cannula which was then manipulated into the thoracic duct. This allowed us to secure the cannula in place with a ligature around the superior lymphatic cannula. The cannula was tunnelled subcutaneously to the left shoulder and brought out through a stab wound. One ml of heparin was injected into the cannula and the end was tied. It was left in place until the next morning. By the above method, it was possible to introduce a cannula as large as 1/8 inch in diameter.

X. Testing for Absorption.

Provided the dogs were healthy, they were tested for absorption seven days after their operation. They were fasted on the day of the test. After suspending each dog on a Pavlov frame, a Foley catheter was introduced into each opening of the Thiry-Vella fistula, and the balloon was inflated to hold each in place. An external balloon adapted on each catheter was inflated close to the skin surface to occlude the fistulous opening (Figure 5). This secured the catheter in place and also prevented

Figure 6. Photograph shows dog being tested on a Pavlov frame with a thoracic duct cannula draining into a collecting tube strapped to the subject's left shoulder.

leaks from the fistula. The test solution was then introduced into each segment of intestine through the Foley catheters which were then sealed off for the duration of the test. Absorption was then followed by one of the following two methods:

(a) Plasma Radioactivity Method (Turner, 1958).

Four ml. of blood were collected from a peripheral vein in an EDTA* tube before the introduction of the test solution and every one hour after the introduction, for eight hours. These samples were centrifuged at 20,000 revolutions per minute (rpm) for 20 minutes. 0.5 ml of plasma was pipetted off from each sample into counting vials, and 2 mls of NCS** solution was added to each to act as a solubilizer. The mixture was gently agitated and left overnight. 10 mls of Fluor*** was then added, and the solution was gently agitated and left overnight again before being counted on a Mark I Nuclear Chicago scintillator. Quenching was corrected by the channels ratio method. The background count was subtracted from each sample.

(b) Lymph Radioactivity Method.

The total amount of lymph draining from the thoracic duct cannula was collected over a maximum period of 8 hours. The total draining each hour was collected in a graduated glass test tube which contained Heparin and was strapped to the left shoulder of the dog (figure 6). 0.5 ml of lymph from each hourly sample was used to count for radioactivity by the same method as for plasma.

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* Ethylene diamine tetra-acetic acid.
** Amersham-Searle, Don Mills, Ontario.
** A solution containing [4 gm 2,5,diphenloxazole and 50 mg p-bis 2-5-phenyloxazolyl] - benzene in solution.

Since the exact amount of lymph collected per hour was known, the total radioactivity collected per hour could be determined.

At the end of each experiment, the fistulae were washed out with 50 ml of normal saline and this was counted for residual radioactivity in the same way as for blood. The plasma radioactivity method was the initial and principal method used to follow absorption. This was done one week and three weeks after surgery, and then every four weeks thereafter. The lymph radioactivity method was used as a terminal procedure except in one batch of group I animals, in which this method was used between one and three weeks after transplantation.

XI. Calculation of Plasma Radioactivity.

This method was adopted from Turner (1958) and was as follows: Blood volume was taken as being 83.2 ml per kilogram of body weight.

$$\text{Plasma volume} = \text{Wt. (kg)} \times 83.2 \times \frac{[\ 100 - \text{hematocrit}\ ]}{[100]}$$

Total plasma radioactivity = radioactivity per ml of plasma x plasma volume.

$$\text{percent radioactivity in the plasma} = \frac{\text{Total plasma radioactivity} \times 100}{\text{Total radioactivity introduced}}$$

XII. Immunosuppression of Control Animals.

Group II and III animals normally received no immunosuppressive therapy. However, after their second test (three weeks after surgery), they were placed on the same immunosuppressive regime as the group I animals for a period of one week and tested again for absorption by the plasma radioactivity method.

XIII. Evaluation of Rejection.

The grafts of all group I animals were examined daily for signs of acute rejection indicated by cyanosis (Preston, et al, 1965). When cyanosis set in, the animals were euthanized and the grafts were examined histologically to confirm the rejection phenomenon as indicated by round cell infiltration, hemorrhage into the mucous membrane and later by sloughing of the epithelium (Preston, et al, 1965). Biopsies of the living graft were also taken periodically and examined for signs of rejection. Postmortem examination was done in every animal that died to determine the cause of death, and histological examination of tissues were done where appropriate.

## RESULTS

### 1. Plasma Radioactivity.

The absorption by Group I, II and III animals were followed by the plasma radioactivity method at one and three weeks after surgery. A graph of total amount of oleic acid present in plasma versus time was plotted for each animal. At one week after surgery, none of the animals showed a maximum level of oleic acid in the plasma within the eight hours. The percentage of oleic acid present in the plasma at eight hours also varied considerably. Thus, in order to compare the absorption between the three groups of animals, the initial rate of absorption was used. This was obtained by measuring the slope of the graph at time zero for each animal. Using this parameter, the mean and standard deviation for each group of animals was obtained. At one week after surgery, these were $1.24 \pm 0.47$ percent per hour for Group I (6 dogs), II (6 dogs) and III (5 dogs) respectively. Statistical comparison of these with the T-test showed no significant difference between the three groups ($p>0.05$).

At three weeks after surgery, the slopes were $1.70 \pm 0.42$, $1.28 \pm 0.33$ and $1.13 \pm .47$ percent per hour respectively for Group I (6 dogs), II (5 dogs), III (6 dogs). Statistical comparison with the T-test showed no significant difference between Groups II and III ($p>0.05$). When Group I was compared with Group II and III, the p. value was less than 0.05. Comparison of these values

at one week with those at three weeks again demonstrated no significant difference ($p>0.05$) except for Group I at three weeks after surgery ($p<0.05$).

These values were similarly calculated for three dogs in Group II and four dogs in Group III after immunosuppression for one week. The values were $1.14 \pm 0.23$ and $1.42 \pm 0.50$ percent per hour respectively. Statistical evaluation showed no significant difference between these two groups and those without immunosuppression ($p>0.05$ in all cases).

The mean and standard deviation of the percentage of plasma radioactivity at each hour were calculated for all groups of animals. The graphs of percent plasma radioactivity versus time are shown on Figure 7 to 14. The results of two long-term survivors in Group I are shown separately on Figures 15 and 16. Their results were not included in the above statistical analysis.

In order to test the possibility of the oleic-9-10-H-3 acid decomposing into H-3 labelled water and becoming absorbed as such, plasma samples from some of these dogs were heated to evaporate the water which was then tested for radioactivity. Negligible radioactivity was noted in the water of each sample, eliminating the possibility that the radioactivity in our animals was due to tritiated water.

II. Thoracic Duct Lymph Radioactivity.

Four animals in Group I, two in Group II and two in Group III were further studied by this method. All animals in Group III,

two in Group II and one in Group I (K122) had previously been studied by the plasma radioactivity method. The other three were new and were studied at one to one and a half weeks after transplantation. The plasma radioactivity was also measured simultaneously in all cases. The results are indicated in Tables 1 and 2. Because of the small number of animals in each group, no statistical evaluations were made.

III. Complications of Surgery.

The chief problem encountered in our transplantation of the intestines was thrombosis of either the artery or the vein. In Group I transplants 43.5 percent of our animals were lost by this complication. This normally occurred within the first two days after transplantation and was indicated by cyanosis and drying of the stomas (Preston et al, 1965). Six of these dogs developed thrombosis between four to eight days after surgery. These dogs either died or were sacrificed and post-mortem examinations confirmed the thrombosis. No clear signs of rejection were noted in these allografts.

Six other animals in Group I developed venous or arterial thrombosis within the first half hour after transplantation. Although the thrombosis could always be relieved by manipulation of the vessels, this was only temporary in every case. These animals were sacrificed.

The incidence of thrombosis in Group II animals was nine percent (1 of 11).

Other complications of surgery included bowel obstruction at the site of anastomosis from which two animals were lost; and hypovolemic shock from which two animals were lost. Two more animals died within 24 hours after surgery and no apparent etiology was demonstrated.

IV. Complications From The Test.

Seven animals were lost from complications directly related to the test. Five of these (three in Group II, one in Group I, and one in Group III), died between 24 to 48 hours after testing. Post-mortem examination in each case revealed pressure necrosis and perforation of a small segment at one end of the intestinal segment with obvious peritonitis, presumably from the bile salt introduced. The only manifestations noted prior to death were anorexia and apparent weakness. The pressure necrosis presumably occurred as a result of compression from the balloons of the Foley catheters, over the eight hours of testing, and this could be reproduced on normal bowel at surgery. In the remaining two animals (one from Group I and one from Group II), direct perforation of the intestinal segment by the catheter was found, resulting in introduction of the test solution directly into the peritoneal cavity. One of these animals died within seven hours and the other within fifteen hours. The test of both these animals were discarded.

V. Complications of Immunosuppressive Therapy.

The most obvious problem encountered with immunosuppression was the delay in wound healing in three Group I animals. This was severe enough to cause evisceration. Examination in all three cases revealed that all the sutures were intact but little evidence of wound healing was noted. Evisceration occurred within six days after surgery.

Four group I animals died from leaks in the intestinal anastomoses. In every case, the suture was found to be intact and no healing had occurred at the site of the leak. These occurred between two and ten days after surgery.

VI. Rejection.

Acute rejection was noted in two of the Group I animals. This was indicated by cyanosis of the stoma. These occurred at seven and 18 days after transplantation. Grossly, these grafts felt soft and the whole wall appeared thin. On histological examination, there was sloughing of the superficial layers of the epithelium with round cell infiltration and hemorrhage in the submucosal areas (Figure 17). The unrejected grafts, however, maintained their smooth pink healthy appearance grossly (Figure 18) with normal mucosa and without round cell infiltration microscopically (Figure 19).

VII. Miscellaneous Causes of Death.

Of the Group I animals, one died from volvulus, one from an overdose of Atrevet, six days postoperatively, four from pneumonia and one from possible distemper indicated by nasal discharge and convulsion. In the latter, however, no attempt was made to confirm the diagnosis at postmortem. One animal in this group was noted to have cyanosis of the graft stoma but when sacrificed, the rest of the graft appeared normal. Two dogs in Group I and two in Group II apparently tore out their grafts postoperatively and were

sacrificed.

VIII. Graft Survival.

In Group I, 13.25 percent of the animals were lost from rejection. The average survival time of these animals was 44.6 days with a range of seven to 96 days. Two animals in this group survived more than six months. One survived 17 months with no evidence of rejection. It appeared well nourished and healthy at the time of death from perforation with the Foley catheter at testing. The second long-term survivor lived seven months and no apparent cause of death was demonstrated. Figures 20 and 21 show the gross appearance of the two long term survivors prior to their deaths. The histological sections of these two animals postmortem are shown in Figures 22 and 23.

The animals in Group I and II without complications, survived indefinitely.

IX. Weight Loss.

This is shown in Table 3.

## DISCUSSION AND CONCLUSION.

### I. Absorption of Oleic Acid.

Various methods of measuring lipid absorption have been carried out in the past. The most popular ones used have been the fecal fat balance method, the serum turbidity method and the plasma and lymph radioactivity method (Wiseman, 1964). The latter two methods were used because they directly measure the amount of absorbed lipid present in the plasma or lymph in contrast to the first two methods which depend on fecal fat recovery and the formation of chylomicrons.

Since lipid absorption depends on several factors such as pH, bile and pancreatic juice, our experiments had to be done under somewhat artificial and ideal conditions in order to control these factors. The solutions were adjusted to optimum pH values between 7 to 7.5, and the same amount of bile was used for each experiment. The effect of pancreatic juice was eliminated by the use of a fatty acid, oleic acid, instead of a triglyceride.

Several investigators in the past have shown that in dogs and in man, the absorption of lipids occurs rapidly in the first few hours, reaching a peak blood value at three to six hours, and then declines slowly (Turner, 1958; Wells et al, 1955; Shingleton, et al, 1955; Baylin et al, 1955; Reemtsma et al, 1957). The mean peak blood level attained varies considerably from three percent to 15 percent depending on the investigator and the method of calculation. In our study, none of the groups one week after surgery

achieved a peak plasma level during the eight hour period. However, at three weeks after surgery, all groups showed a peak plasma level at five to six hours. These results agree well with those of previous investigators. The mean peak plasma value attained by our animals was between 3.5 to 5.5 percent.

This is comparable to those obtained by Turner (1958) from whom the method of calculation was adopted, but lower than those obtained by other (Wells, et al, 1955; Shingleton, et al, 1955; Baylin, et al, 1955; Reemtsma, et al, 1957), who used whole blood instead of plasma for radioactivity counts.

Two dogs in Group II and one in Group I produced considerable amounts of secretions from their grafts during their tests. Each secreted between 400 and 500 cc in the eight hours of testing compared to the usual 50 to 100 cc normally seen in the other animals. This amount of secretion probably diluted our test solution and the results of these animals showed a slower rate of absorption. One dog in Group II at one week after surgery showed an amount of radioactivity that was considerably higher than that found in the other dogs of the same group. This was felt to be due to a technical error in counting because of the great amount of color quenching appearing in the samples. The results from this animal were eliminated from our calculations.

The dose of 250 microcuries was selected after several smaller doses tried on normal animals failed to produce a level of radioactivity readily distinguishable from background in our

samples. The dose appears high compared to the 50 microcuries used by some investigators (Wells et al, 1955; Shingleton et al, 1955; Baylin et al, 1955; Reemtsma et al, 1957). These latter investigators, however, used $I^{131}$ which gives a much higher efficiency in counting than $H^3$. Previous investigators have had to use up to 750 microcuries of $H^3$ (Russell et al, 1971).

II. Effect of Immunosuppression.

In order to study the effect of immunosuppression on our absorption tests, Group II and Group III animals were placed on a week of immunosuppressive therapy. Our results indicate that the course of immunosuppression did not affect the absorption of oleic acid. The absorption curves (figures 13,14), appeared similar to those without immunosuppression. The initial rate of absorption indicated by the asymptotes of the graphs at time zero were $1.14 \pm 0.23$ and $1.42 \pm 0.5$ percent per hour respectively in Group II and III. These were not significantly different from those obtained before immunosuppression ($p>0.05$).

The most obvious effect of immunosuppression was wound disruption. This agrees well with the known effects of the immunosuppressives which inhibit protein synthesis and cause delay in wound healing. No doubt part of this is due to our lack of knowledge of the optimum dose of immunosuppressives required, and this complication could be eliminated with further knowledge of the immunosuppressives. One peculiar phenomenon was observed with

immunosuppression. In almost all Group I animals, the plasma was noted to be of yellowish color. Although this was initially thought to be due to hemolysis of blood samples, this was never observed in Group II or III dogs without immunosuppression even though some of the latter animals were studied at the same time as the Group I animals with the same technique for collecting blood samples. Using immunosuppression, this phenomenon was noticed in two animals in Group II and two in Group III. The bilirubin, serum glutamic oxaloacetic transaminase were normal in all cases. The liver of three of these Group I animals, which died within a week after this phenomenon was observed, appeared grossly normal and microscopic examination showed no evidence of cholestasis which Starzl et al (1967) noted with Prednisone and antilymphocyte serum.

III. Rejection.

This was noticed in a total of seven animals only. No valid figure for the incidence of rejection among Group I animals can be obtained due to the numerous animals lost from other complications. Presumably, some of these would have developed rejection had they lived long enough. In all seven cases, only a host against graft reaction was observed. The graft versus host reaction (GVHR) observed by previous investigators (Idezuki, et al, 1968; Ruiz, and Lillehei, 1972) was not noted. In all Group I animals that died, the liver appeared normal on gross and histological examination. However, the mesenteric lymph nodes were enlarged in all

cases as noted by others (Idezuki et al, 1968; Ruiz and Lillehei, 1972). The antilymphocyte serum used in Group I animals probably suppressed the GVHR as well as the host versus graft reaction. This effect of antilymphocyte serum has been demonstrated by Brent et al, (1967). The antilymphocyte serum was initially tested by hemagglutination and the titre was found to be 1:528. The titre of the leucoagglutination test, however, does not correlate well with graft survival (Jeejeebhoy, 1967). Thus no consistent further testing was done as the method of preparation of the serum remained the same throughout.

IV. Route of Transport.

Since Bloom and Chaikoff's experiments (Bloom et al, 1950; 1951a; 1951b; Chaikoff et al, 1951), the transport of long chain fatty acids has been presumed to be predominantly by the lymphatics. However, several studies have shown that the portal venous system normally transports up to 30 percent of long chain fatty acids after absorption. (Gallagher et al, 1965; Dawson et al, 1964; Hyun et al, 1967). This route may be even more important in cases of defective esterification (Koldovsky et al, 1963). Our studies show that even after the thoracic duct has been cannulated, a considerable amount of oleic acid still entered the blood (Tables 1 and 2). The oleic acid in this case could pass from the lymphatics to the blood by the lymphatic venous anastomoses described previously (Job, 1918; Silvester, 1912; Lee, 1920), or it could, as previously found, pass

directly into the portal system. Due to the small number of animals done, no statistical comparison could be made between the absorption by these animals with and without thoracic duct cannulation. Thus, although no definite conclusion can be drawn in this regard, our results would seem to indicate that the portal route of transport is important for oleic acid.

V. Lymphatic Regeneration.

Goott et al (1960) and Kocandryle et al (1966) using radiopaque dye injected into the lymphatics, concluded that lymphatic regeneration of allografted and autografted intestine does not occur before two weeks after transplantation. Our studies with thoracic duct cannulation show a significant amount of oleic acid (17.8 percent in K208) in the thoracic duct lymph as early as one week after allografting (Tables 1 and 2). The oleic acid could conceivably come from the venous system through lymphatico venous anastomoses. However, the fact that a greater amount was recovered from the lymph than from the blood would mitigate against this explanation. One allograft (J1311), however, showed almost negligible oleic acid in its lymph. This could be due to lack of lymphatic regeneration or to poor lymph recovery as the great majority of the lymph was recovered in the first hour of the test. It would appear, therefore, that lymphatic regeneration is present before two weeks after allografting in some dogs, although no definite conclusion about the average time of regeneration can be drawn due to the small number of subjects studied.

Our experiment differs from those of Goott et al (1960) and Kocandryle et al (1966) in that we re-anastomose the lymph nodes at surgery. This might have aided lymphatic regeneration. Another explanation of the results would be that the lymphatics at one week are still too small to be demonstrated by lymphangiograms although they are open to allow free passage of lipids. Our initial lymphangiograms on a few allografts at four weeks after transplantation demonstrated that the methylene blue dye does outline the lymphatics (Figure 18) and after injection of lipoidal into the lymphatics (Figure 24), the lymphangiograms show that the dye crossed the mesenteric lymph nodes.

VI. Nerve Regeneration.

Although this present study was not designed to study nerve regeneration, some interesting observations were noted. During testing of animals, all in Group III (in which no denervation was done) showed obvious discomfort when the internal balloons of the Foley catheters were inflated. However, this was not seen in any of Group I or II animals at one and three weeks after transplantation, probably indicating denervation. Interestingly, three months after transplantation, one dog in Group II began to show the discomfort seen in Group III animals. Of the long term survivors, in Group I, one showed this effect at about one year while the second did not show it at all after six months. If the discomfort on balloon inflation can be taken as an indication of sensory re-inervation of the ileum, the autografts showed nerve

regeneration as early as three months after transplantation. This is a gross method of evaluation and would have to be verified by histological examination.

VII. Vascular Thrombosis.

Comparison of the incidence of vascular thrombosis between Group I and Group II shows a great difference. We feel that the most important cause of the thrombosis in Group I animals is the frequent discrepancy in the caliber of vessels used in our anastomoses. This no doubt hindered perfect end to end anastomosis. This effect, of course, was not present in Group II dogs. One could also postulate a reaction of the host toward the graft causing a hypercoagulability state.

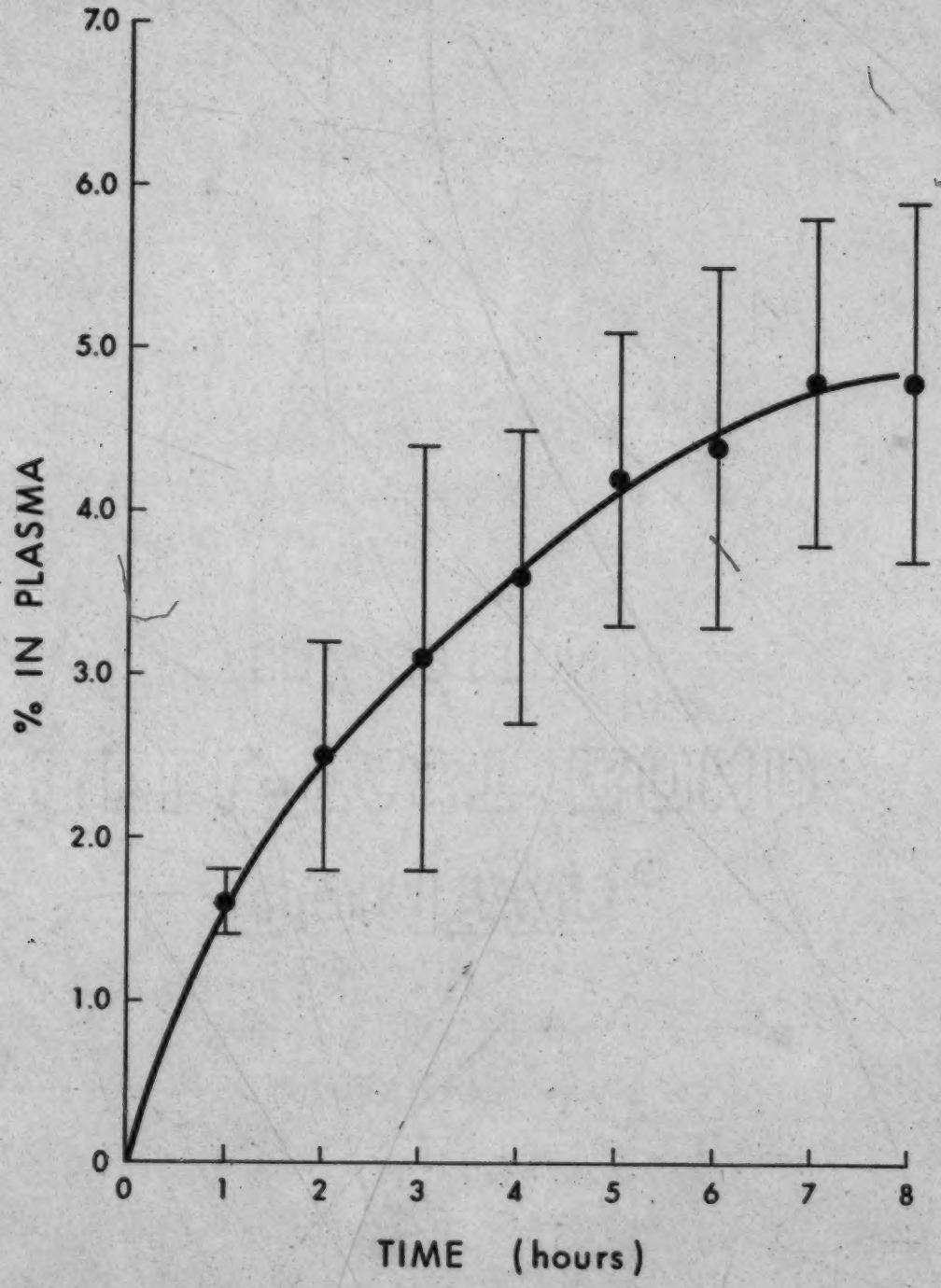


Figure 7. Group I animals one week after transplantation. Graph of time versus plasma $H^3$ level expressed as a percentage of the total dose introduced (6 dogs), showing the mean value of the standard deviation at each hour for figures 7 - 14.

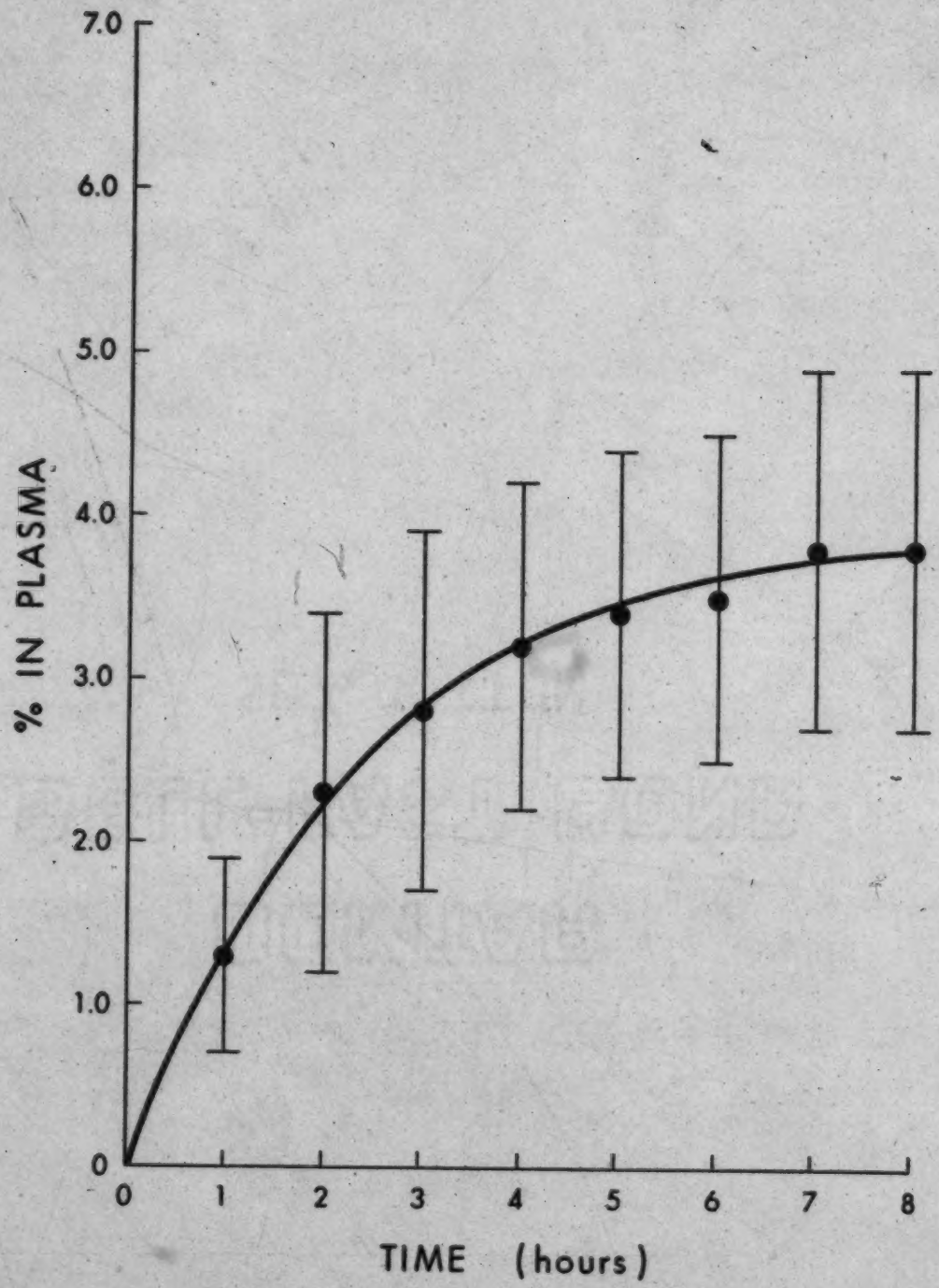


Figure 8. Group II animals, one week after transplantation without immunosuppression. Graph of time versus plasma $H^3$ level expressed as a percentage of the total dose introduced (6 dogs).

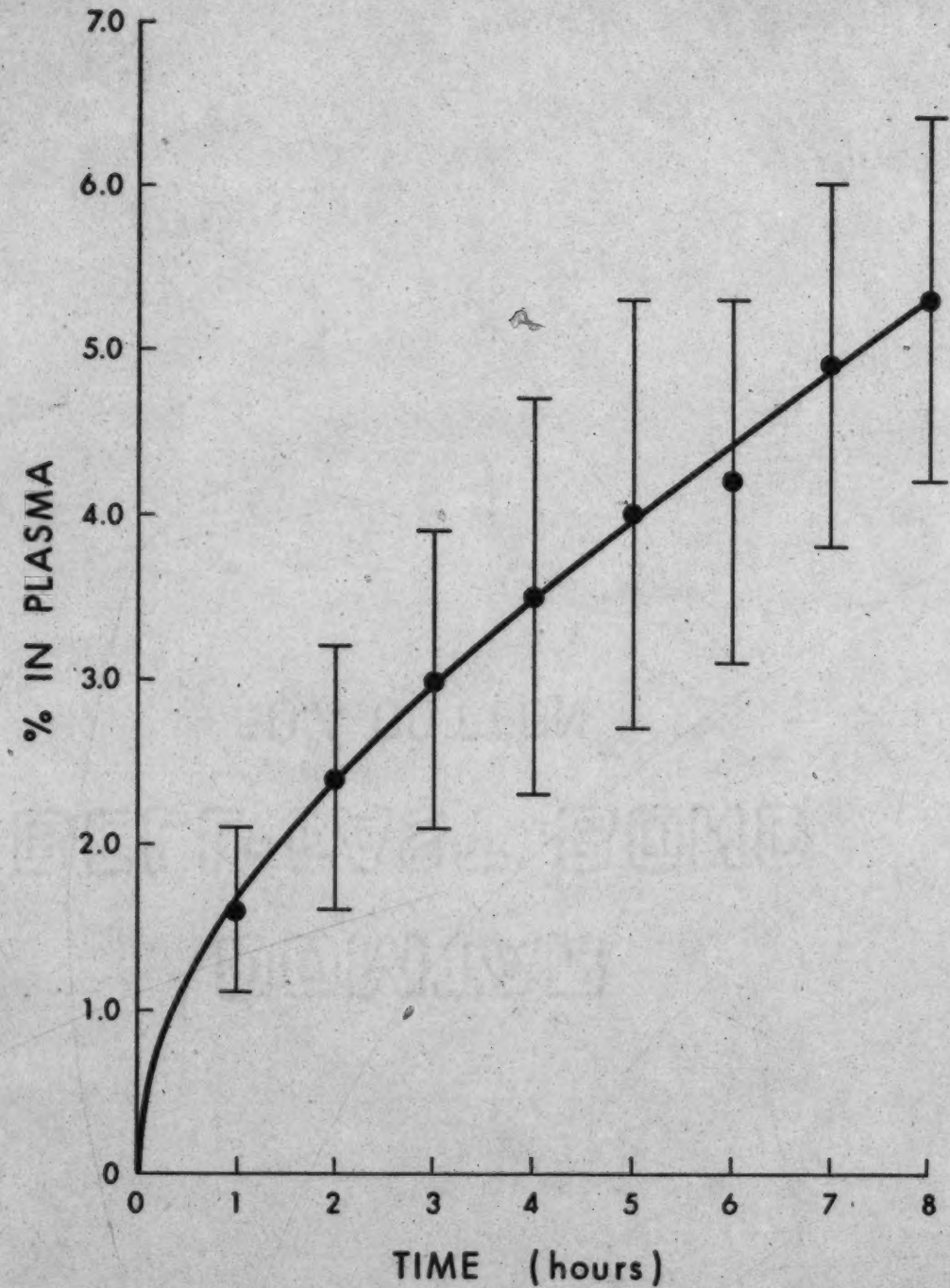


Figure 9. Group III animals, one week after surgery without immunosuppression. Graph of time versus plasma $H^3$ level expressed as a percentage of the total dose introduced (6 dogs).

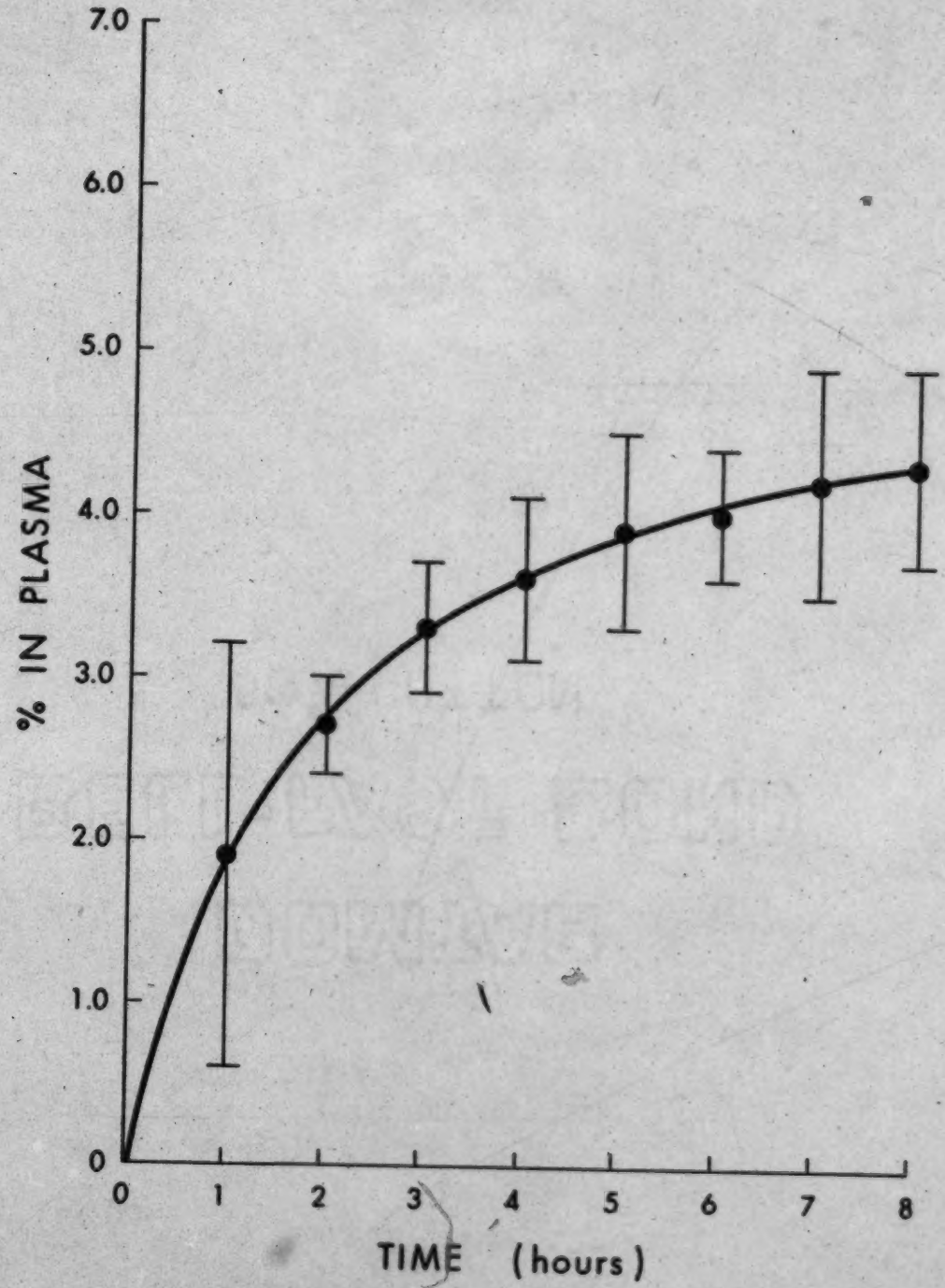


Figure 10. Group I animals, three weeks after transplantation. Graph of time versus plasma $H^3$ level introduced as a percentage of the total dose introduced (6 dogs).

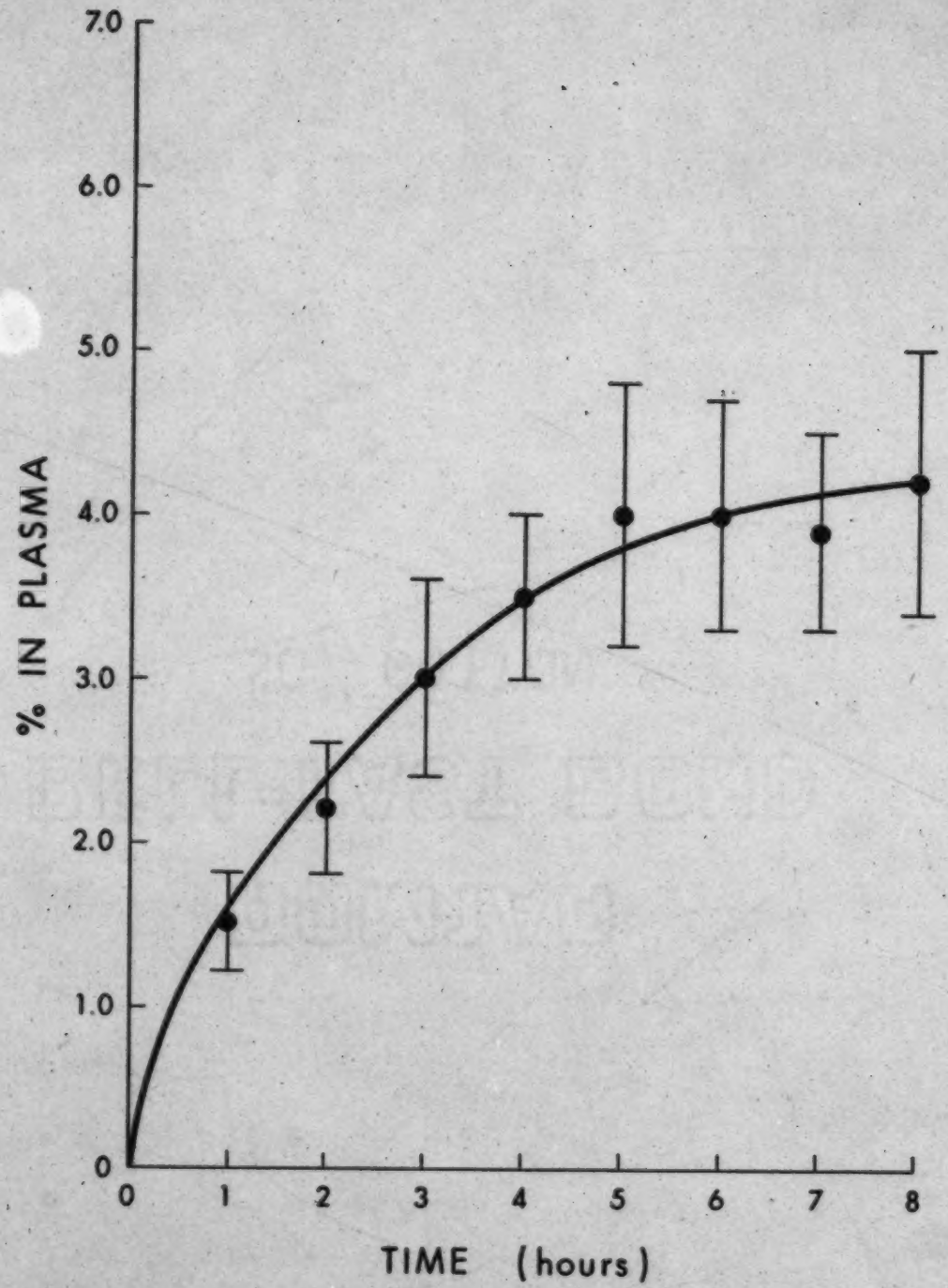


Figure 11. Group II animals, three weeks after transplantation without immunosuppression. Graph of time versus plasma $H^3$ level expressed as a percentage of the total dose introduced (5 dogs).

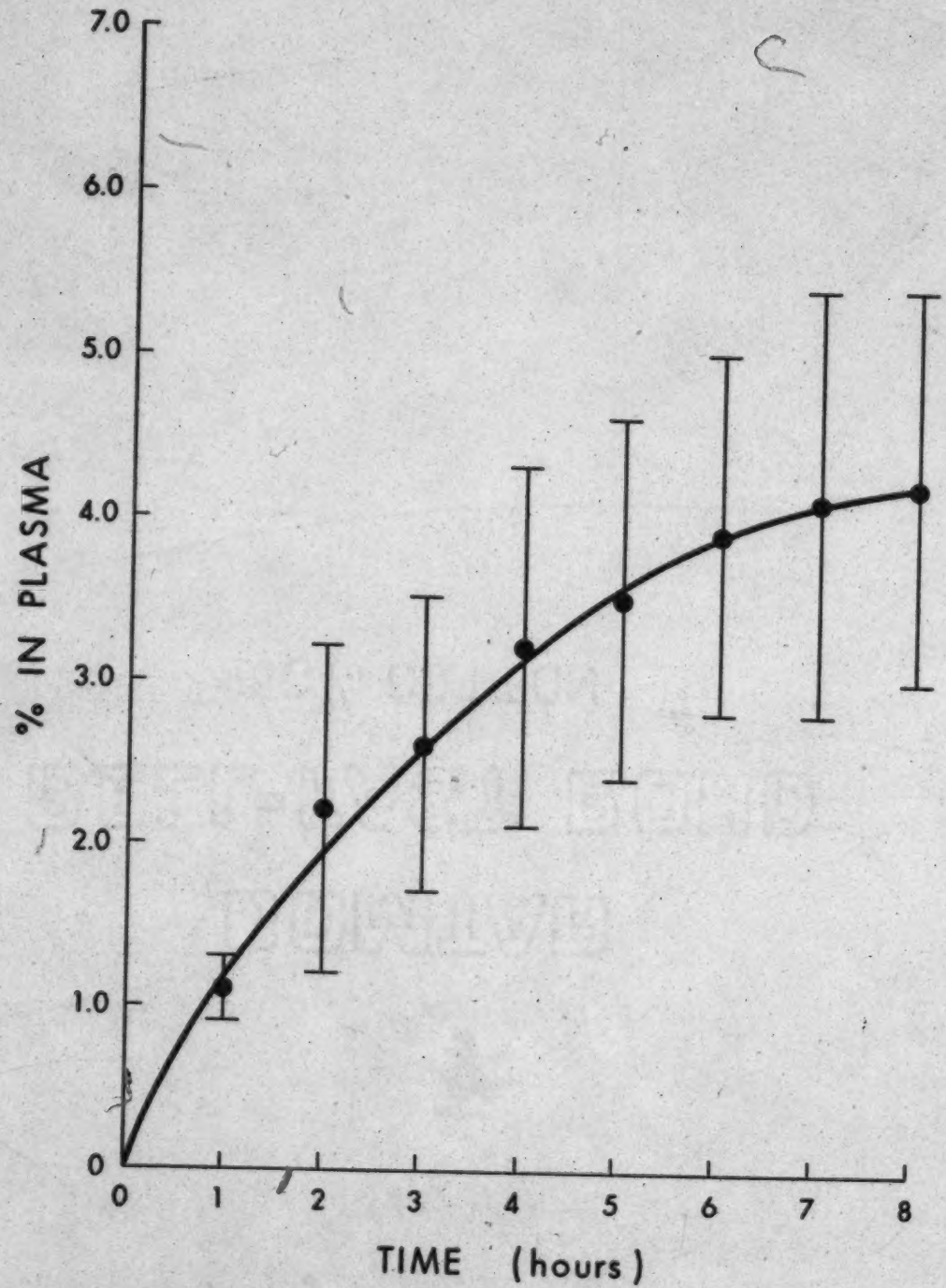


Figure 12. Group III animals, three weeks after surgery without immunosuppression. Graph of time versus plasma $H^3$ level expressed as a percentage of the total dose introduced (6 dogs).

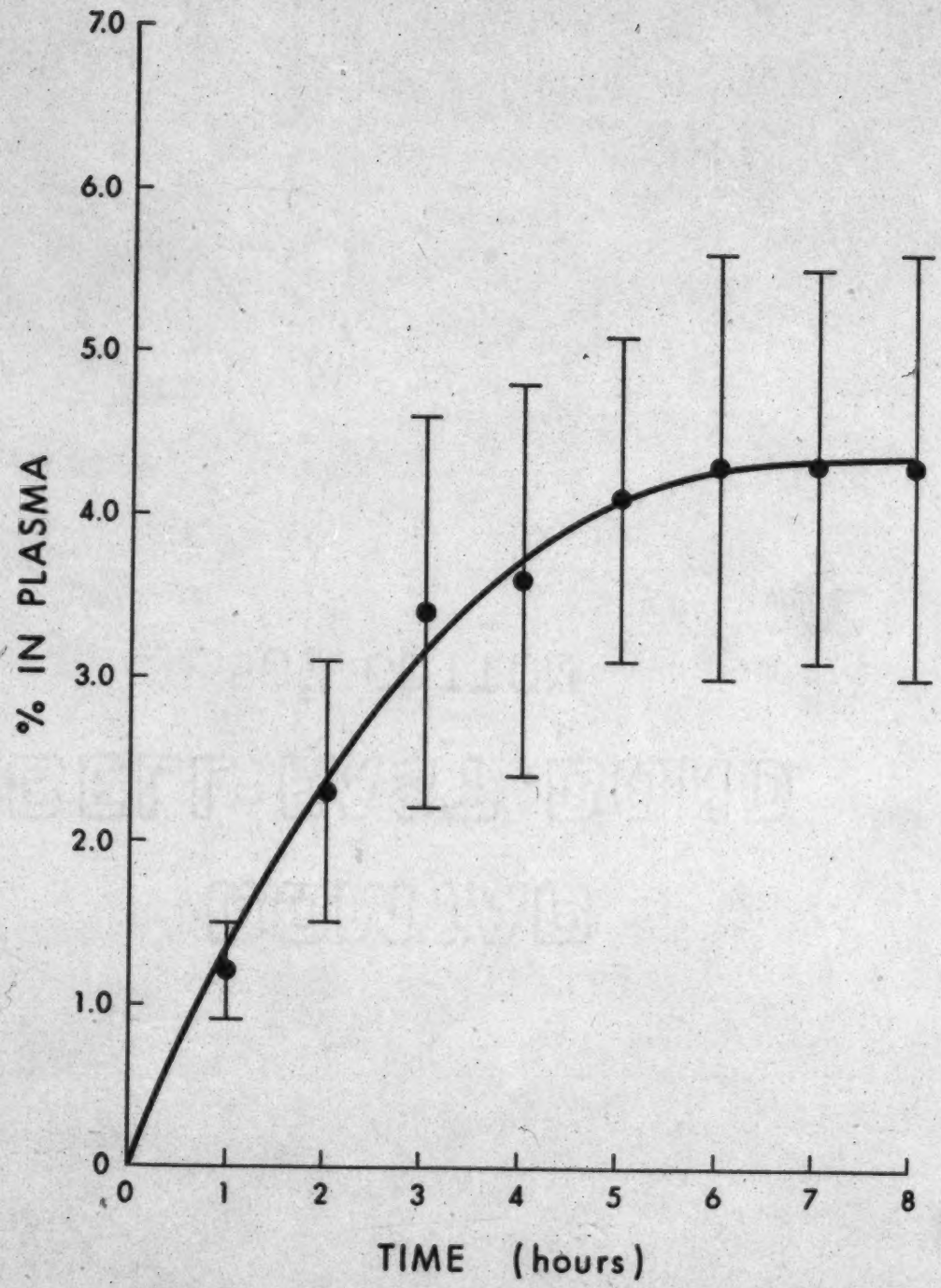


Figure 13. Group II animals, four weeks after transplantation and after a one week course of immunosuppressive therapy. Graph of time versus plasma $H^3$ level expressed as a percentage of the total dose introduced (3 dogs).

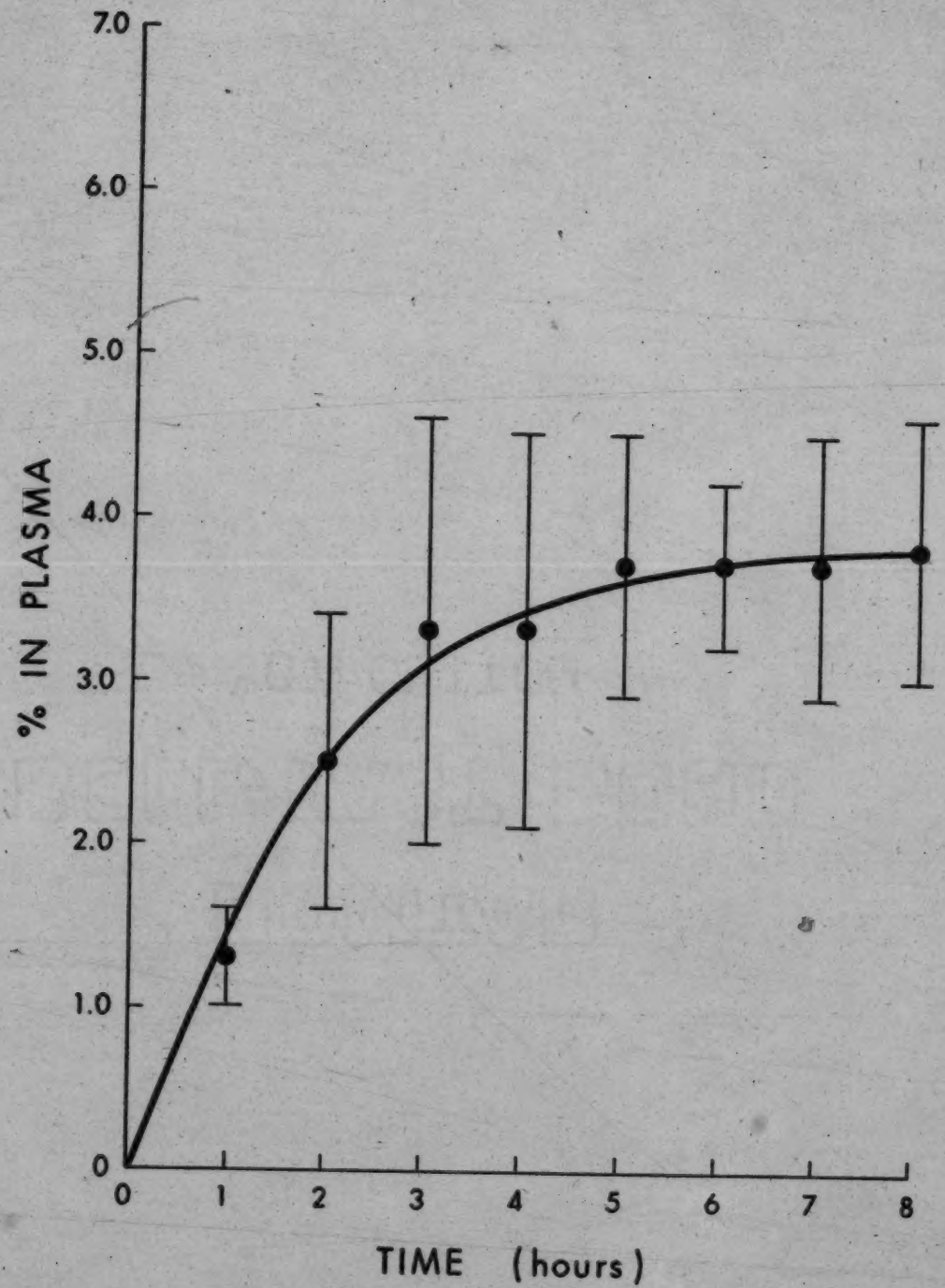


Figure 14. Group III animals, four weeks after surgery and after a one week course of immunosuppressive therapy. Graph of time versus plasma $H^3$ level expressed as a percentage of the total dose introduced (4 dogs).

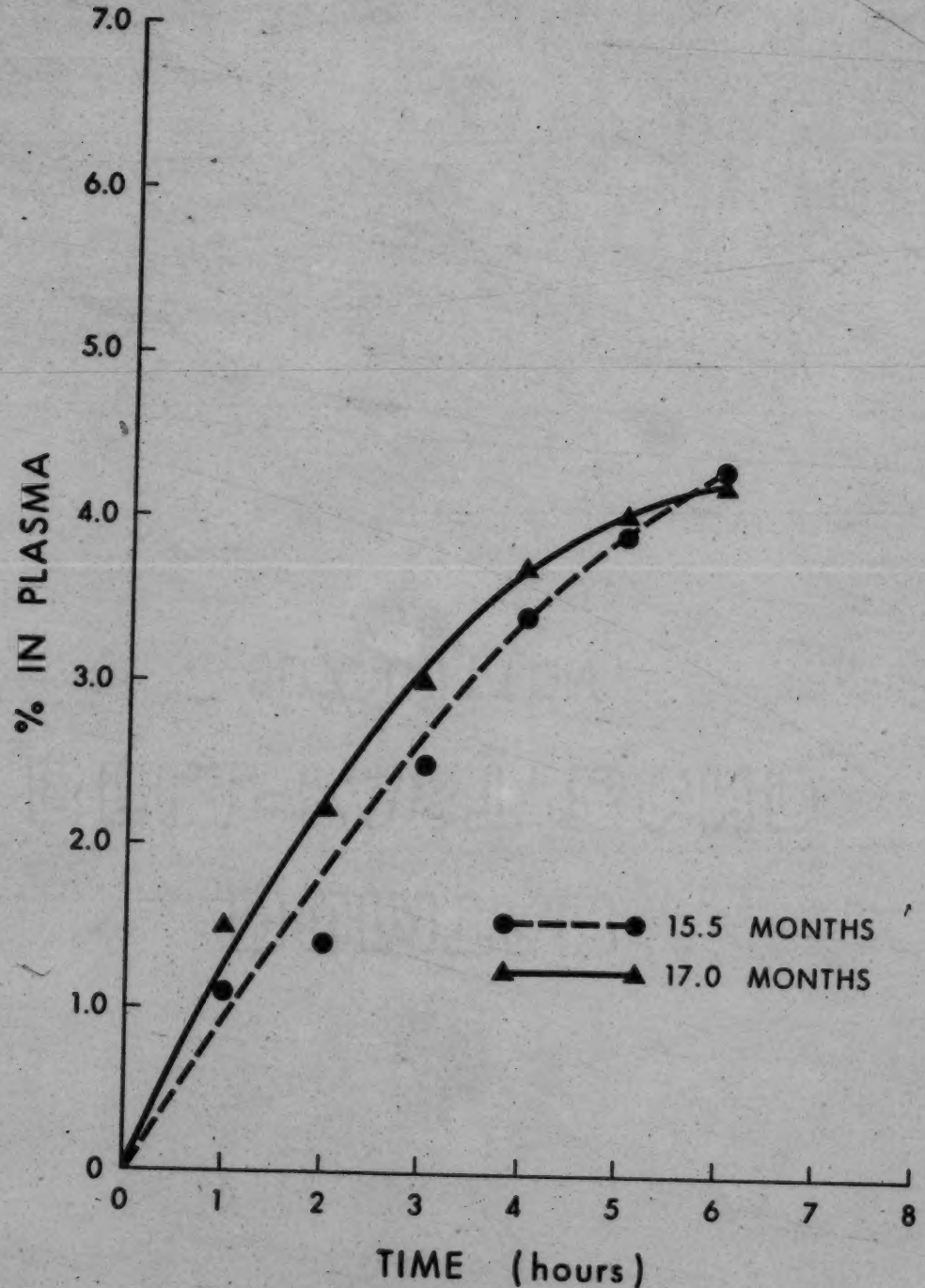


Figure 15. Long term allograft H386. Graph of time versus plasma $H^3$ level at various times after transplantation.

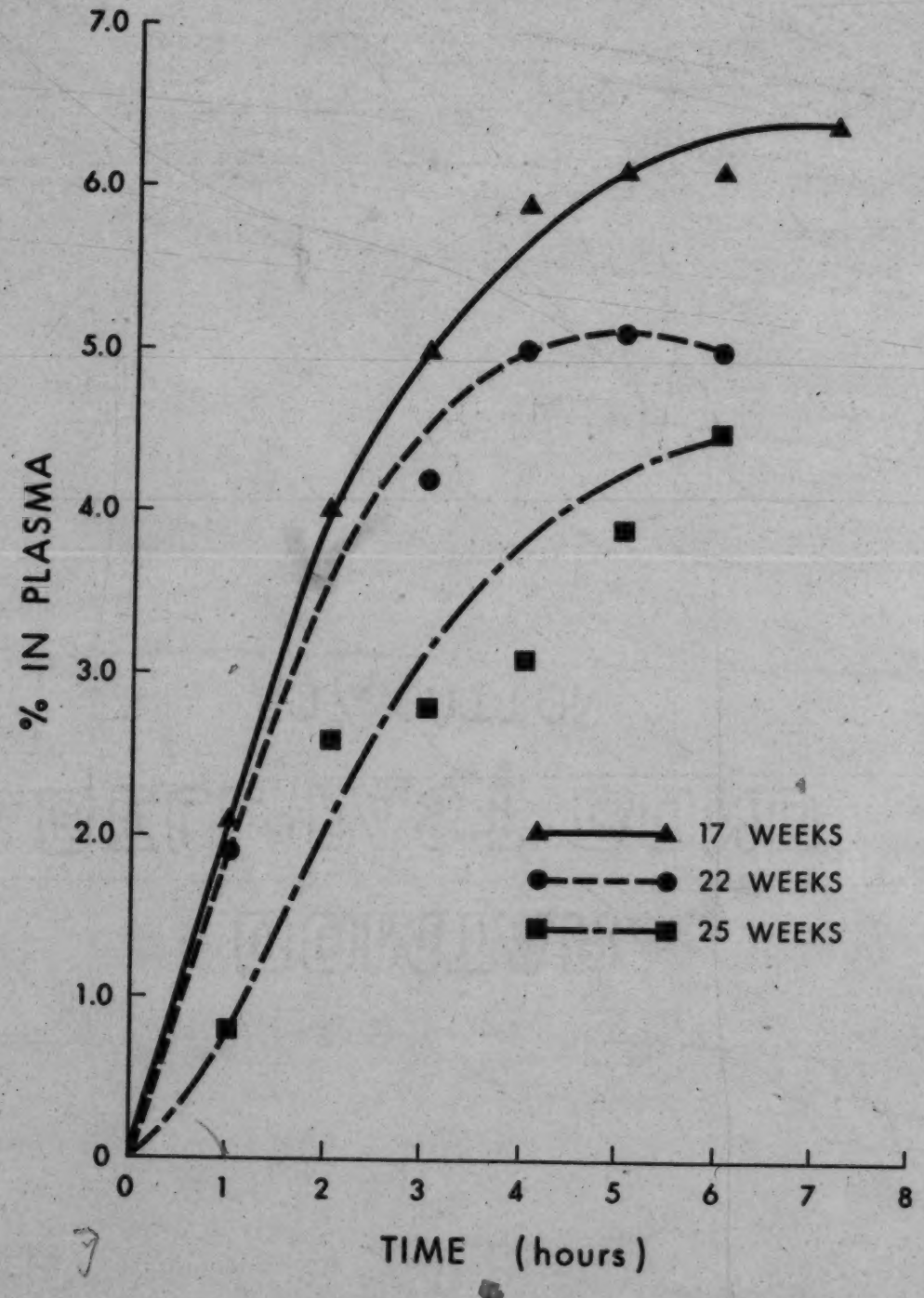


Figure 16. Long term allograft J250. Graph of time versus plasma $H^3$ level at various times after transplantation.

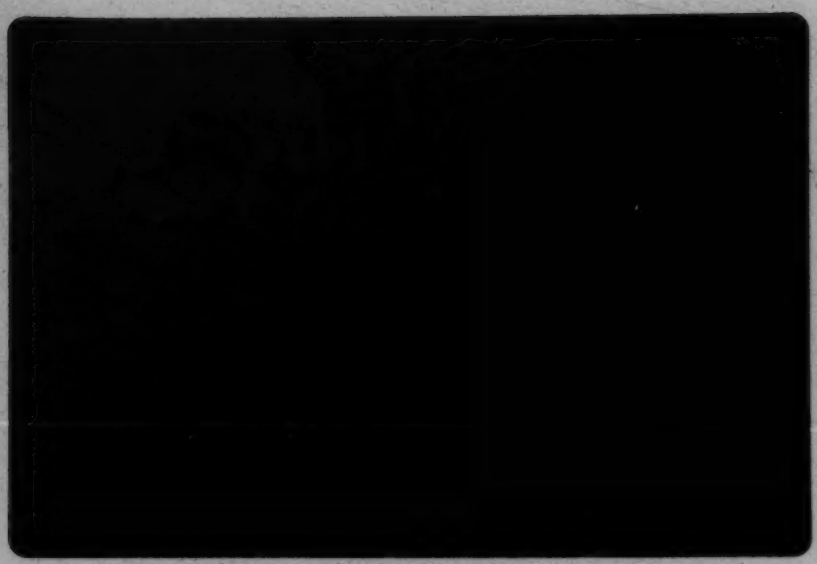

Figure 17. Microscopic section of rejected allograft showing heavy round cell infiltration in the submucosal areas with loss of epithelium.

Figure 18. Photograph shows viable graft four weeks after transplantation. The graft is smooth, pink, with normal vessels. Note the presence of lymphatics as outlined by the methylene blue dye.

Figure 19. Microscopic section of allograft biopsy showing healthy mucosa without round cell infiltration.

Figure 20. Photograph of long term allograft H386, sixteen and one half months after transplantation. The graft is pink, with a normal thickness of the wall and normal appearing mucosa.

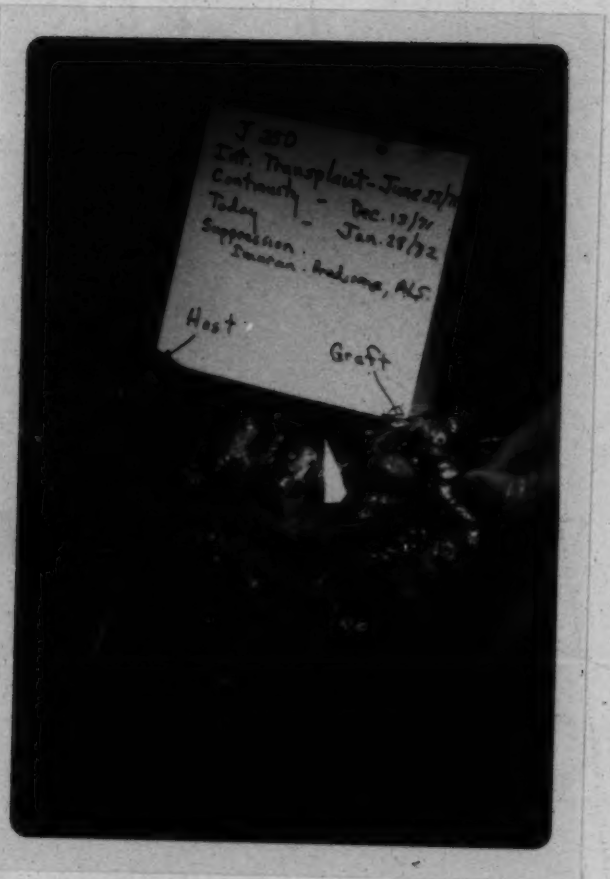


Figure 21. Photograph of long term allograft J250, seven months after transplantation. The graft shows very little difference from the host intestine. Both are covered with adhesions. The graft has been placed back in continuity with the host intestine and white arrows indicate the points of anastomoses.

Figure 22. Microscopic section of long term allograft H386, sixteen and one half months after transplantation. The mucosa is well preserved with little round cell infiltration.

Figure 23. Microscopic Section of long term allograft J250, seven months after transplantation. Note the preservation of the height of the mucosa and little round cell infiltration.

Figure 24. Photograph of allograft showing Lipoidal being injected into a lymphatic vessel previously outlined with methylene blue dye.

FIGURE 25. LYMPHANGIOGRAM SHOWING DYE CAUSING MESENTERIC LYMPH NODES.

| TYPE | GROUP I | | | GROUP II | | | GROUP III | |
|---|---|---|---|---|---|---|---|---|
| TIME POST OP | 3 wks | 1 wk | 1.5 wk | 1 wk | < six weeks > | | | |
| DOG NO. | K122 | J1311 | J1348 | K208 | J1680 | K118 | J1689 | K24 |
| 1 hr | 4.0 | 2.1 | 3.19 | 2.1 | 1.1 | 0.9 | 0.9 | 1.0 |
| 2 hrs | 5.8 | 3.2 | 3.3 | 2.4 | 2.5 | 2.1 | 1.7 | 1.3 |
| 3 hrs | 6.5 | 3.7 | 3.5 | 2.9 | 2.9 | 2.7 | 2.2 | 1.6 |
| 4 hrs | 6.5 | 4.03 | 3.5 | 3.0 | 3.5 | 3.0 | 2.5 | 2.0 |
| 5 hrs | 6.58 | 4.2 | 4.0 | 3.14 | 3.5 | 3.3 | 2.6 | 2.7 |
| 6 hrs | 6.4 | 4.1 | 4.5 | 3.4 | 3.5 | 3.6 | 3.10 | 2.9 |
| 7 hrs | 6.4 | | 4.8 | 3.5 | 3.65 | 3.75 | 3.17 | |
| 8 hrs | 6.4 | | 4.8 | 3.6 | | 3.9 | 3.67 | |

Table 1. Total plasma radioactivity level (Percent of total dose introduced) at each hour, for Group I, II and III, taken while thoracic duct cannulated.

| TYPE | GROUP I | | | GROUP II | | | GROUP III | |
|---|---|---|---|---|---|---|---|---|
| TIME POST OP | 3 wks | 1 wk | 1.5 wk | 1 wk | < six weeks > | | | |
| DOG NO. | K122 | J1311 | J1348 | K208 | J1680 | K118 | J1689 | K24 |
| 1 hr | 0.4 | 0.1 | 1.1 | 0.4 | 0.06 | 0.3 | 0.1 | 0.47 |
| 2 hrs | 0.82 | 0.13 | 1.6 | 5.1 | 0.1 | 0.6 | 0.7 | 4.16 |
| 3 hrs | 1.57 | 0.14 | 2.7 | 11.0 | 1.74 | 5.4 | 1.9 | 6.56 |
| 4 hrs | 3.3 | n/l* | 3.8 | 13.5 | 3.18 | 8.0 | 3.57 | 11.7 |
| 5 hrs | 4.05 | | 4.7 | 15.3 | 3.88 | 9.8 | 4.35 | 13.15 |
| 6 hrs | 5.1 | | 5.2 | 16.3 | 4.1 | 10.6 | 4.78 | 13.2 |
| 7 hrs | 5.6 | | 6.3 | 17.2 | 4.28 | 11.7 | 5.38 | c/a** |
| 8 hrs | 5.7 | | 7.0 | 17.8 | c/a** | 12.6 | 5.8 | |
| TOTAL VOL. LYMPH | 368 cc | 110 cc | 364.5 cc | 465.5 cc | 110.7 cc | 254 cc | 213 cc | 100.5 cc |

* no lymph
** cannula out

Table 2. Thoracic duct lymph radioactivity at each hour (percent of total dose introduced) for Group I, II and III.

| GROUP | AVERAGE WEIGHT LOSS AT ONE WEEK (% BODY WEIGHT | AVERAGE WEIGHT LOSS AT THREE WEEKS (% BODY WEIGHT |
|---|---|---|
| I | 15.0 | 21.5 |
| II | 5.8 | 6.3 |
| III | 7.8 | 9.0 |

Table 3. Weight loss of dogs at one and three weeks after Surgery.

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